1.0. SOP FOR READING AND REPORTING BLOOD SMEARS

SOP Title: SOP for Reading and reporting Blood smears
SOP Author(s):
Implementation Date: _____
Document received by: _____ _____ _____
Date Signature
Agreement to comply with this SOP: _____ _____
Date Signature

1.1 Definition
This SOP is used when taking fingerpick blood, reading and reporting blood smears

1.2 Responsible team member
Laboratory technicians

1.3 Materials:
Light microscope
Sterile lancet
Glovesfilter paper
Funnel
Graduated measuring cylinder
Geimsa stock soln.
Buffer PH 7.2
Cotton wool
Toilet rollers
Lens paper
Absolute methanol
Hair drier
Laboratory timer
Anisole or oil immersion
Slide rack
Slide boxes
Slide tray
Drying rack
Staining jar
Pipette
BLOOD SLIDE RECORDING sheet
2 Tally counters

1.4 Task
Preparation of materials, collection of finger prick blood smears, microscopic examination parasite count, slide storage and reporting of results through out the study period

1,4.1 Prior to initiation of the study
The Laboratory technicians should:

- Be interested in the scientific aspects of the study and
- Have sufficient time free from other obligations to prepare and conduct the diagnosis
- Make sure that the procedures stated in the study protocol are applicable in his/her center and fully understood.

- Make sure that the physical location and facilities are sufficient to allow the study to be undertaken efficiently..
- Adequate equipment/facilities for subject follow-up, examination and care.
- Adequate facilities for product storage. Adequate facilities for laboratory assay.
- Discuss the procedures in detail with the investigator

1.4.2 During the study

1. Greet mother/ care giver
2. Select preferred site for pricking
3. Clean the area using wet cotton swabs soaked with methylated spirit.
4. **Always remember** to wipe **dry** the spirit using a dry swab before pricking to avoid fixing of thick smear
5. Prick the site gently using sterile disposable lancet.
6. Immediately dispose the pricker into sharps disposable container.
7. Quickly, wipe off the first drop and take drops for thick and thin smear by allowing the microscope slide to just touch blood drop while paying attention for the slide not to come into direct contact with patient's finger
8. Prepare both thick and thin smears in a single slide
9. Stain blood smears appropriately
10. Examine the slides Make sure that the slides to be examined are *completely* dry.
11. Write your name, the date and the slide number of slide reading (ie 1 or 2) on the BLOOD SLIDE RECORDING sheet.
12. Take the first slide and write the laboratory number, PIN number, study subjects initials and date written on the slide into the first rows on the BLOOD SLIDE RECORDING sheet.
13. Read the thick film first and followed thin film to identify species.
14. Perform parasite counting
15. For negative slides report **NPS –** meaning ***No Parasites Seen.***
16. Record the result in the BLOOD SLIDE RECORDING sheet.
17. When the slides are read store slides and give BLOOD SLIDE RECORDING sheet to the nurse immediately

2. 0. ACQUISITION OF SPECIAL SKILLS FOR LABORATORY CONDUCT

2.*1* ***Fingerpick blood sample collection***

For infants/neonates the preferred site for pricking is the heel but pricking at the tip of great toe may also be convenient for sample collection. In adults and older children a finger prick at the tip of third finger counted from the thumb (middle) of the left hand is recommended site for pricking.

The procedure is performed aseptically by cleaning the area using wet cotton swabs soaked with 70% ethanol. **Always remember** to wipe **dry** the spirit using a dry swab before pricking to avoid fixing of thick smear. Prepare the blood lancet to be used taking

precaution not to contaminate its sharp end. Apply gentle pressure to make the finger tip tense for easy pricking. Prick the site gently but firm enough for you to be able to achieve good flow of capillary blood. Immediately dispose the lancet into sharps disposable container. Working quickly, wipe off the first drop and take drops for thick and thin smear by allowing the microscope slide to just touch blood drop while paying attention for the slide not to come into direct contact with patient's finger. Proceed with preparation of thick and thin smears as shown below.

2.2 Thick smear preparation

This is prepared by putting one-two drops of finger prick blood close to the frosted end of the slide. Use the corner of another slide to help you spread blood uniformly to make a bigger drop of about 2cm diameter. The standard thick smear should allow one to be able to read letters underneath the smear.

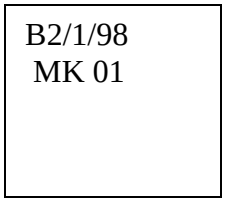
2.3 Thin smear preparation

This involves collection of a small drop of blood at few centimeters from the thick smear. Using another slide (spreader), touch a drop of blood with the spreader inclined at an angle of 45^0 and then spread the blood along the first slide at steady quick movement to make the thin smear.

Allow the slide to air dry in a flat surface taking precaution to ensure that it is free from dust and flies.

2.4 Sample labeling

Traditionally, the labeling may consist of patient initials, unique patient identification number, and date of sample collection. For Bahir Dar, all samples collected will start with "B" or "N" for Nazrette sites respectively. The "K" or "S" letter will be followed by date of sample collection The second line of labeling will bear the initials of study infants (MK for Moges Kassa) followed by patient's identification number (PIN).



2.5 Staining

Check that stain has passed quality control (known positive and negative slides have been checked)

Giemsa stain at a concentration of 10% will be used and the staining time will be 30 minutes. Arrange the slide in the staining jar and add Giemsa solution until the slides are completely submerged. Once staining time is over, wash to remove the staining using clean water and arrange the slides in the slide racks for them to air dry.

1. Fix the thin film by adding 3 drops of methanol or by dipping it in container of methanol for a few seconds
2. Place the slides back in staining dish

3. Gently pour 3% or 10 % Giemsa's working solution into the dish until the slide are totally covered
4. Allow staining for 30-45 minutes (for 3% Giemas's working solution or 10 minutes (for 10% Giemsa's working solution.
5. Pour clean water gently I n the dish to float off iridescent scum on the surface of the stain
6. Gently pour of the remaining stain, and rise again in clean water for a few second pour the water off
7. Wipe the back of each slide
   Place the slide in the draining rack

2.6 Examination of blood films

Apply a drop of immersion oil or anisole to an area of the film, examine for malaria parasites with 100 times objective lens.

- The microscopist will examine 200 high power fields (HPF) before the slide is declared negative.
- The microscopist will record the number of asexual parasites per 200 White Blood Cells (WBC) and the number of sexual forms per 500 WBC
- For negative slides report **NPS –** meaning ***No Parasites Seen.***

2.7 Counting malaria parasites

18. 2 hand tally counters are required to count parasites and WBC separately.
19. After 200 WBC have been counted record the result in the BLOOD SLIDE RECORDING sheet.
20. If you count 500 asexual parasites before counting before counting 200 WBC. Stop the count and record the figure.
21. Convert this figure into the number of asexual parasites per 200 WBC format (see formula below) before recording in the BLOOD SLIDE RECORDING sheet.

$$\frac{\text{Number of asexual parasites}}{\text{Number of WBCs counted}} \times 200 = \text{Number of asexual forms/200 WBC}$$

2.8 Things you should do for the microscope (Dos)

1. Cover the microscope when not in use to avoid dust (use plastic cover or clean cloth)
2. Protect microscope from fungal growth by keeping it in worm cupboard fitted with heaters
3. After each day's wok, clean the emulsion oil from x100 objective lens with a soft cloth dampened in xyelene and polish with a clean cloth
4. Clean the lens with lens cleaning tissue or soft clean cloth
5. When carrying, support the microscope by holding the base and board tube
6. During transportation, pack the microscope in the box

2.9 Thing you should not do (the don'ts)

1. Dismantle the microscope without being trained
2. Using a piece of cloth with oil emulsion to clean the lens
3. Cleaning the painted part of the microscope with alcohol
4. Leaving the lens part empty

Hemoglobin (Hb) estimation using HemoCue

We shall be using the HemoCue machine for Hb estimation. The machine gives the results after 30-50 seconds. The microcuvettes are use for collection of the blood sample to be measured and then it is loaded into the machine for result determination. Precaution should be taken to avoid contaminating the part that allows light to pass through. While working, care should be taken to ensure that no air bubbles develop when blood is being drawn into the cuvettes, as this will give the incorrect estimation of Hb. Once the Hb reading is displayed out, record the reading in appropriate form and discard the cuvette.

GOOD LUCK